



*Available Online through*

**www.ijptonline.com**

## **INFLUENCE OF PHYTOMINERAL SORBENTS ON METABOLISM CHANGE IN INFLAMMATORY INJURY OF RAT TISSUE**

**Andrey A. Shaposhnikov\***, Ulyana A. Krut, Natalya G. Gabruk, Elena A. Shentseva, Dmitriy E. Smalchenko

Belgorod State University, 308015, 85 Pobedy st., Belgorod, Russia.

Email: [Shaposhnikov@bsu.edu.ru](mailto:Shaposhnikov@bsu.edu.ru)

*Received on 05-06-2016*

*Accepted on 27-06-2016*

### **Abstract**

One of the most perspective remedy for the treatment of purulent wound is sorbtion and biological active substance. They studied the action of montmorillonite-containing clays (hereinafter MCC), which was modified by thyme's extract (*Thymus serpyllum*) or phytomineralsorbent, as a dry powder or in the form of gel on biochemical changes in the tissues of the rats with skin-muscle injuries. It was shown that the positive dynamics of indicators characterizing the pH, the concentration of total protein and glucose in the exudates of inflammatory wounds with the sorption of active composites reduced the activity of the inflammatory process and contributed to a better regeneration of damaged tissues. This metabolite may indicate the degree of tissue injury and the intensity of inflammation in the tissues, which is useful for the diagnosis of inflammatory injuries.

**Key words:** Montmorillonite-containing clays, Phytomineral sorbents, Rats, Inflammatory injury, Purulent exudate, concentration of total protein and glucose, exudates pH.

### **Introduction**

Wound damages and subsequent pyoinflammatory processes refer to the most frequent pathologies in surgery requiring the study and the improvement of treatment methods [1-3]. Early healing is based on biochemical processes in injuries and in other tissues, particularly in blood [4-6]. This publication discusses the changes of purulent exudate active acidity, the concentration of crude protein and glucose in it.

The study of pH in the wound is an important indicator of wound healing. The content of unoxidized metabolic products which contribute to the severity of the disease increases in the focus of inflammation as the result of metabolic disorders [7]. Acidity which reflects the quality and sometimes the effect of drugs solutions has a great importance for the action of antiseptics and disinfectants [8].

pH values of effusion fluids are used in clinical practice for the transition diagnosis of serous exudate into seropurulent one. A generally accepted boundary is the pH value equal to 7.20.

The determination of total protein concentration in effusions is the main moment in the study of festering wounds. At a light damage of vessels the center of inflammation is infiltrated only with low molecular albumins. During a more severe damage exudate demonstrates high molecular globulins, and finally, the largest molecules of fibrinogen, turning into fibrin tissue. A common value discriminating exudate from transudate, is the protein content in the serum of the biological material which should be over 25 g/cm<sup>3</sup> [9].

In the case of tissue integrity violation the content of cells is released, including glucose, which is one of the most important metabolism components in a body. The presence of glucose in the effusion fluids creates a favorable environment for the development of pathogenic microflora. There are no studies on the content of glucose in the effusion fluids from the festering wounds at the moment. The concentration of glucose in the exudate or the transudate of purulent wounds can be correlated with the concentration of glucose in blood, due to its exit from the blood vessels. These indicators describe the inflammation level and the prerequisites for the development of proliferation processes concerning tissue surface achieved during an acute phase. The monitoring of these parameters is necessary during the obtaining and the practical use of new composites in purulent surgery. The aim was to determine the dynamics of protein concentration, glucose, and pH in the exudate during an acute phase of the wound healing process.

## **Materials and methods**

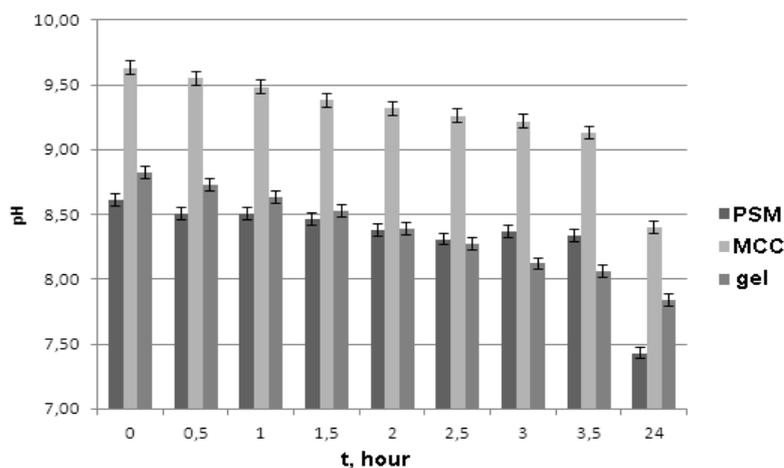
The experiment was performed using 50 Wistar rats. Animals were modeled with pyoinflammatory wounds in an interscapular region: using a purse-string suture a port was sewn in which *Escherichia coli* was introduced infecting  $2 \cdot 10^8$  of microbe bodies in a fixed dose [10]. The surgery of animals was performed under anesthetic: 300 mg/kg of chloral hydrate were administered intraperitoneally. All studies were performed in compliance with the requirements of the "European Convention for the protection of vertebrate animals used for the experimental and other scientific purposes" (Strasbourg, 1986). Depending on the treatment experimental animals were divided into five groups. In the first group the dressing of a wound was performed using an isotonic NaCl solution (0.9%); an aqueous tincture of thyme was used in the second group; a complex scheme of treatment was used in the third group, which were included in the first phase of wound healing (7 days), the dry form of purified MCC (montmorillonite-containing clays), and in the second phase included the helium form of MCC. In the fourth group of experimental animals the

dry form of phytomineral sorbents (PMS) was used for dressings during the first phase of wound healing process and PMS gel form was used during the second phase. In the fifth group of rats gel form PMS dressing were performed throughout the whole period of wound healing process. Metabolic dynamics in the tissues with chronic inflammatory injury was performed during an acute phase of wound healing in abundantly exudating wounds on the following parameters: pH, the concentration of total protein and glucose in the exudate.

The sampling was performed from the wound surface (by the method of an average sample selection) into special vials with lids. pH was determined by potentiometric method using pH meter Metter-Toledo AG (Switzerland). Besides, the determination of isotonic solution pH and thyme extract pH determination was performed. Total protein concentration was determined spectrophotometrically according to the biuret reaction, using the Specord device 210 Plus (Germany). The glucose concentration was determined by titrimetric method according to Hagedorn-Iesen [9]. The statistical analysis of results was performed using the licensed software MS Excel XP. The reliability of differences was determined according to Student's t-criterion. The level of significance  $p < 0.05$  was taken as an authentic one.

### Results and discussion

In order to compare the pH of purulent exudate and the influence of phytomineral sorbents on the focus of inflammation, we studied the aqueous extracts of experimental samples initially: PMS, MCC (montmorillonite-containing clays) and PMS gel form (gel). In order to obtain the isotherms of water extract pH values concerning the studied samples the samples were prepared in the ratio of 1:50 (sample: water). During the first 3.5 hours the indicators were recorded every 30 minutes, then the sample was left for 1 day. Fig. 1 shows the average values of aqueous extract pH in the samples under study.



**Fig. 1. Evaluation of aqueous extract pH in the studied samples during the experiment. (Baseline indicator of water pH: 6.64).**

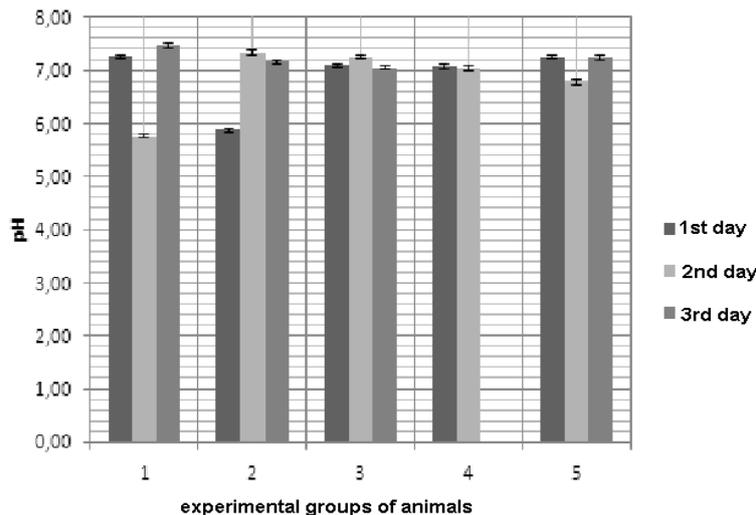
Thus, according to the experimental data, MCC (montmorillonite-containing clays) has more basic medium of an aqueous solution.

We performed a series of purulent exudate pH indicator measurements from model skin and muscle wounds of albino rats (Fig. 2).

The obtained data during the first day of purulent inflammation process suggest that the rats of the II-nd, III-rd and IV-th experimental group with the characteristic features of festering, have a slightly acidic or a neutral pH ranged from 5.88 to 7.09, which is below the boundary pH criterion equal to 7.20. This aspect suggests the violation of acid-base balance of cells and tissues and as the result the acidosis.

During the second day PH values of purulent exudate were lower than the boundary criterion among the animals of I-st, IV-th and V-th experimental group by 20, 2.2 and 6%, respectively. The most pronounced acidosis was observed in the I-st group.

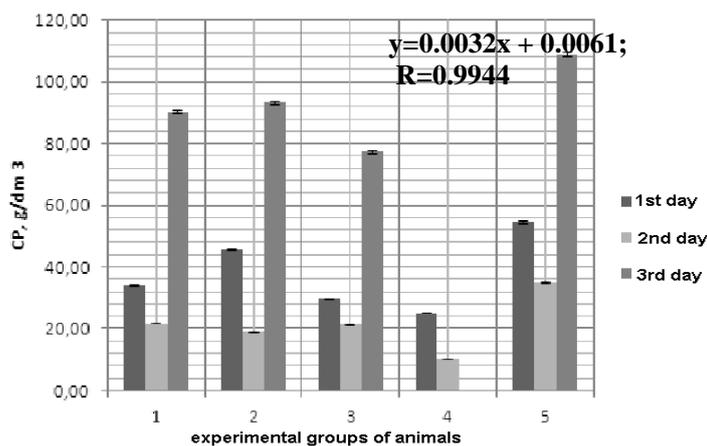
During the third day purulent exudate pH changed towards an acidic value in the III-rd and the II-nd experimental group of animals, despite the fact that the pH of thyme extract and MCC (montmorillonite-containing clays), which performed the treatment in these groups of animals, had a weakly basic character. It should be noted that there was no exudation in the IV-th experimental group. Also there were no sharp fluctuations in the pH values of purulent exudate within this group of animals, which may indicate a stable course of the disease (Fig. 2).



**Fig. 2. Dynamics of pH value changes in the samples of purulent exudate during the experiment.**

Thus, the performed studies concerning the acidity of purulent wounds, pH exudates in particular, showed a significant acidification of media, regardless of a dressing tool choice. However, the experimental group of rats, where the wound dressing was carried out by the dry form of PMS, exudation stopped on the third day of the experiment and the dynamics of pH smooth changes was observed.

The data analysis on protein content in wound exudate was demonstrated on Fig. 3.

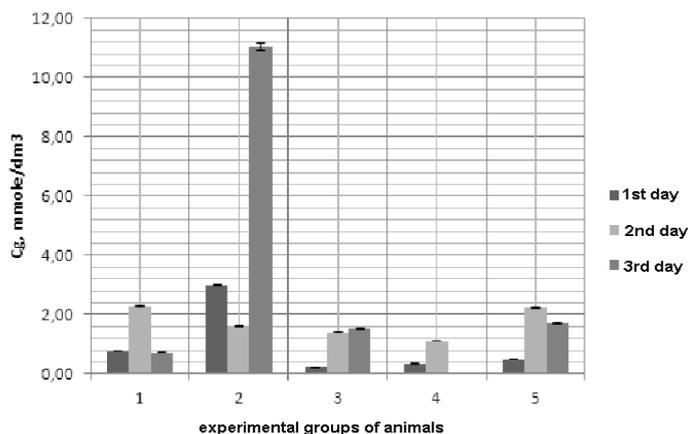


**Figure 3. Changes of total protein concentration in the exudate of a purulent wound ( $\lambda=547$  nm).**

The results showed that the I-st, II-nd, III-rd and V-th groups of animals had the total protein concentration increase in a wound exudate. By the third day of the experiment the protein content in these groups made more than  $25 \text{ g/cm}^3$ , which means that the exudate from the wound is characterized as an exudate. In the IV-th experimental group total protein concentration had a negative trend. Effusions stopped on the third day of the experiment. On the first day of the experiment, the protein concentration in the exudate was  $25 \text{ g/cm}^3$ , and on the second day it made  $10 \text{ g/cm}^3$ , which is smaller than the conventional value for the exudate.

The indicators of glucose concentration in the wound are presented on Figure 4.

Based on the results of the study, the experimental groups showed an uneven change in glucose concentration. The glucose concentration changed in waves among the rats of the I-st, IV-th and V-th experimental groups: it was increased on the second day of the experiment and it was reduced on the third day of the experiment. In the II-nd and the III-rd group the glucose concentration decreased on the second day and rose on the third day. At that, the concentration of glucose increased 4 times by the 3-rd day as compared to the first ones in the II-nd experimental group.



**Fig. 4. Glucose concentration dynamics in the exudate of a purulent wound.**

Thus, it can be concluded that the use of PMS during the exudation phase of wound healing process reduces the total protein concentration that favors the reduction of the inflammatory process and the formation of the regeneration processes.

The concentration of glucose in a purulent wound exudate is proportional to the concentration of glucose in blood. This metabolite may indicate the degree of tissue injury and the intensity of inflammation in the tissues, which will be useful in the diagnosis of inflammatory injuries.

## References

1. Dário, G.M., Silva Da, Gonçalves D.L., Silveira P., Junior A.T., Angioletto E., Bernardin A.M., 2014. Evaluation of the healing activity of therapeutic clay in rat skin wounds *Materials Science and Engineering*, 43: 109–116 (in English).
2. Dawson, J.I., Oreffo R.O.C., 2013. Clay: New opportunities for tissue regeneration and biomaterial design. *Advanced Materials*. 25 (30): 4069–4086 (in English).
3. Shai, A. Wound healing and ulcers of the skin. Diagnosis and therapy - the practical approach / A. Shai, H.J. — Springer-Verlag Berlin Heidelberg, 2005. — 279 p.
4. Grose, R., S. Werner, 2004. Wound-healing studies in transgenic and knockout mice (Review). *Applied Biochemistry and Biotechnology - Part B Molecular Biotechnology*, 28 (2): 147 –166 (in English).
5. Kumbhare, D., Parkinson W., Brett Dunlop R., Adili A., 2012. Biochemical Measurement of Injury and Inflammation in Musculoskeletal Surgeries. *Orthopedic Surgery*. 9: 166 –182 (in English).
6. Buhanov, V.D, Shaposhnikov A.A., Vezentsev A.I., Klochkova G.N., Okhrimchuk D.P., Krut U.A., 2014. Comparative analysis of biochemical and morphological indices of blood of rats in the third stage of the process in the treatment of wound healing phytomineralsorbents. *Scientific statements BSU. Series Medicine Pharmacy*. 11 (182): 177 – 180 (in Russian)
7. Kunz, C.R, 2004. Intrapleural injection of transforming growth factor-antibody inhibits pleural fibrosis in empyema:1636 –1344(in English).
8. Yushkov, B.G., Urakov A.L., Tadjiev R.I., Larionov L.P., Zabokritsky N.A., Krivopalov S.A., 2010. Acidity index purulent exudates in the chemical and pharmacological study. *Biomedicine*: 148-150 (in Russian).
9. Hodyukova, A.B., Baturevich L.V., 2011. Laboratory examination of exudative fluids. *Medical News.*: 17-19 (in Russian).

10. Sendryakova, V.N., Kokaeva I.K., Troch K.A., Brutkin M.V., 2013. Purulent wounds modeling issues for rats:

Sat. Materials of Intern. scientific. Conf. 8: 38 (in Russian).

**Corresponding Author:**

**Andrey A. Shaposhnikov\***,

**Email:** [Shaposhnikov@bsu.edu.ru](mailto:Shaposhnikov@bsu.edu.ru)